

WE CLAIM:

*but G 67* 1. A method for accelerating the rate of mucociliary clearance in a subject in need of such treatment comprising administering to the subject an effective mucociliary clearance stimulatory amount of a composition comprising a Kunitz-type serine protease inhibitor and a physiologically acceptable carrier.

2. The method according to claim 1, wherein the composition is administered to the lung airways.

3. The method according to claim 1, wherein said composition is administered directly by aerosolization.

4. The method according to claim 1, wherein said composition is administered directly as an aerosol suspension into the mammal's respiratory tract.

5. The method according to claim 4, wherein said aerosol suspension includes respirable particles ranging in size from about 1 to about 10 microns.

6. The method according to claim 4, wherein said aerosol suspension includes respirable particles ranging in size from about 1 to about 5 microns.

7. The method according to claim 4, wherein said aerosol suspension is delivered to said subject by a pressure driven nebulizer.

8. The method according to claim 4, wherein said aerosol suspension is delivered to said subject by an ultrasonic nebulizer.

9. The method according to claim 4, wherein said aerosol suspension is delivered to said subject by a non-toxic propellant.

10. The method according to claim 1, wherein said carrier is a member selected from the group consisting of a physiologically buffered solution, an isotonic saline, normal saline, and combinations thereof.

*but G 68* 11. The method according to claim 1 wherein the Kunitz-type serine protease inhibitor is aprotinin.

*but G 68* 12. The method according to claim 1, wherein the Kunitz-type serine protease inhibitor comprises the amino acid sequence:

MAQLCGL RRSRAFLALL GSLLLGVLA -1

ADRERSIHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50

YLTKEECLKK CATVTEENATG DLATSRNAAD SSVPSAPRRQ DSEDHSSDMF 100

NYEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 150

35 ACMLRCFRQQ ENPPLPLGSK VVVLAGLFVM VLILFLGASM VYLRVARRN 200

QERALRTVWS SGDDKEQLVK NTYVL 225

(SEQ ID NO.: 49).

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13. The method according to claim 1, wherein the Kunitz-type serine protease inhibitor comprises the amino acid sequence:

5 ADRERSIHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50  
YLTKEECLKK CATVTENATG DLATSRNAAD SSVPSAPRRQ DSEDHSSDMF 100  
NYEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 150  
ACMLRCFRQQ ENPPLPLGSK VVVLAGAVS 179  
(SEQ ID NO.: 2)

MLR AEADGVSRLL GSLLLGVLA -1  
ADRERSIHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50  
YLTKEECLKK CATVTENATG DLATSRNAAD SSVPSAPRRQ DSEDHSSDMF 100  
NYEEYCTANA VTGPCRASPP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 150  
ACMLRCFRQQ ENPPLPLGSK VVLAGLFVM VLILFLGASM VYLIRVARRN 200  
QERALRTVWS SGDDKEQLVK NTYVL 225  
(SEQ ID NO.: 45),

MAQLCGL RRSRAFLALL GSLLLSGVLA - 1  
20 ADRERSIHDF CLVSKVVGRC RASMPRWWYN VTDGSCQLFV YGGCDGNSNN 50  
YLTKEECLKK CATVTENATG DLATSRNAAD SSVPSPARQQ DSEDHSSDMF 100  
NYEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 150  
ACMLRCFRQQ ENPPLPLGSK VVVLAGLFVM VLILFLGASM VYLIRVARRN 200  
QERALRTVWS FGD 213  
25 (SEQ ID NO.: 47),

ADRERSIHDF	CLVSKVVGRC	RASMPRWY	VTDGSCQLFV	YGGCDGNSNN	50	
YLTKEECLKK	CATVTENATG	DLATSRNAAD	SSVPSAPRRQ	DSEDHSSDMF	100	
NYEEYCTANA	VTGPCRASFP	RWYFDVERNS	CNNFIYGGCR	GNKNSYRSEE	150	
30	ACMLRCFRQQ	ENPPLPLGSK	VVVLAGLFVM	VLILFLGASM	VYLIRVARRN	200
	QERALRTVWS	SGDDKEQLVK	NTYVL		225	
	(SEQ ID NO.: 70),					

and

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ADRERSIHDF CLVSKVVGRC RASMPRWYN VTDGSQLFV YGGCDGNSNN 50  
YLTKEECLKK CATVTENATG DLATSRNAAD SSVPSAPRRQ DSEDHSSDMF 100

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NYEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 150  
ACMLRCFRQQ ENPPLPLGSK VVVLAGLFVM VLILFLGASM VYLIRVARRN 200  
QERALRTVWS FGD 213  
(SEQ ID NO.: 71).

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*Dwlr E3*  
14. The method according to claim 1, wherein the Kunitz-type serine protease inhibitor comprises the amino acid sequence:

10 IHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50  
YLTKEECLKK CATV 64  
(SEQ ID NO.: 4),

15 CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50  
YLTKEECLKK C 61  
(SEQ ID NO.: 5),

20 YEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 150  
ACMLRCFRQ 159  
(SEQ ID NO.: 6),

25 CTANAVTGPC RASFPRWYFD VERNSCNNFI YGGCRGNKNS YRSEE 150  
ACMLRC 156  
(SEQ ID NO.: 7),

30 IHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50  
YLTKEECLKK CATVTENATG DLATSRNAAD SSVPSAPRRQ DSEDHSSDMF 75  
NYEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 125  
ACMLRCFRQ 159  
(SEQ ID NO.: 3),

35 CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50  
YLTKEECLKK CATVTENATG DLATSRNAAD SSVPSAPRRQ DSEDHSSDMF 100  
NYEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 150  
ACMLRC 156  
(SEQ ID NO.: 50),

ADRERSIHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 25  
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YLTKKEECLKK CATVTENATG DLATSRNAAD SSVPSAPRQQ DSEDHSSDMF 75  
NYEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 125  
ACMLRCFRQQ ENPPLPLGSK VVVLAGAVS 179  
(SEQ ID NO.: 1),

5

and

ADRERSIHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN

50

10 YLTKEECLKK CATVTENATG DLATSRNAAD SSVPSAPRQQ DSEDHSSDMF  
100

NYEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE  
150

ACMLRCFRQQ ENPPLPLGSK 170

15 (SEQ ID NO.: 52).

15. The method according to claim 1, wherein the Kunitz-type serine protease inhibitor comprises the amino acid sequence:

20 ADRERSIHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50  
YLTKKEECLKK CATVTENATG DLATSRNAAD SSVPSAPRQQ DS 92  
(SEQ ID NO.: 8).

16. The method according to claims 12, 13, 14 or 15, wherein the Kunitz-type serine protease inhibitor is glycosylated.

17. The method according to claims 12, 13, 14 or 15, wherein the Kunitz-type serine protease inhibitor contains at least one intra-chain cysteine-cysteine disulfide bond.

30 18. The method according to claims 12, 13, 14, or 15, wherein the Kunitz-type serine protease inhibitor contains at least one intra-chain cysteine-cysteine disulfide bond selected from the cysteine-cysteine paired groups consisting of CYS11-CYS61, CYS20-CYS44, CYS36-CYS57, CYS106-CYS156, CYS115-CYS139, 35 and CYS131-CYS152, wherein the cysteine residues are numbered according to the amino acid sequence of native human placental bikunin.

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19. Use of a Kunitz-type serine protease inhibitor in manufacturing a medicament for accelerating the rate of mucociliary clearance in a subject in need of such treatment.

5 20. Use according to claim 19, wherein the Kunitz-type serine protease inhibitor is in a form suitable for administration to lung airways.

21. Use according to claim 19, wherein the Kunitz-type serine protease inhibitor is in a form suitable for administration by aerosolization.

10 22. Use according to claim 19, wherein the Kunitz-type serine protease inhibitor is in a form suitable for administration as an aerosol suspension into the mammal's respiratory tract.

23. Use according to claim 22, wherein said aerosol suspension includes respirable particles ranging in size from about 1 to about 10 microns.

24. Use according to claim 22, wherein said aerosol suspension includes respirable particles ranging in size from about 1 to about 5 microns.

15 25. Use according to claim 22, wherein said aerosol suspension is generated by pressure driven nebulizer.

26. Use according to claim 22, wherein said aerosol suspension is generated by an ultrasonic nebulizer.

20 27. Use according to claim 22, wherein said aerosol suspension includes a non-toxic propellant.

28. Use according to claim 19, wherein medicament includes a carrier selected from the group consisting of a physiologically buffered solution, an isotonic saline, normal saline, and combinations thereof.

25 29. Use according to claim 19, wherein the Kunitz-type serine protease inhibitor is aprotinin.

30 30. Use according to claim 19, wherein the Kunitz-type serine protease inhibitor comprises the amino acid sequence:

MAQLCGL RRSRAFLALL GSLLLGVLA -1

ADRERSIHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50

YLTKEECLKK CATVTENATG DLATSRNAAD SSVPSAPRRQ DSEDHSSDMF 100

NYEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 150

ACMLRCFRQQ ENPPLPLGSK VVVLAGLFVM VLILFLGASM VYLIRVARRN 200

QERALRTVWS SGDDKEQLVK NTYVL 225

(SEQ ID NO.: 49).

35 31. Use according to claim 19, wherein the Kunitz-type serine protease inhibitor comprises the amino acid sequence:

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AGSFLAWL GSLLLGVLA -1  
ADRERSIHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50  
YLTKEECLKK CATVTENATG DLATSRNAAD SSVPSAPRRQ DSEDHSSDMF 100  
NYEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 150  
5 ACMLRCFRQQ ENPPLPLGSK VVVLAGAVS 179  
(SEQ ID NO.: 2),

MLR AEADGVSRLL GSLLLGVLA -1  
ADRERSIHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50  
10 YLTKEECLKK CATVTENATG DLATSRNAAD SSVPSAPRRQ DSEDHSSDMF 100  
NYEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 150  
ACMLRCFRQQ ENPPLPLGSK VVVLAGLFVM VLILFLGASM VYLIRVARRN 200  
QERALRTVWS SGDDKEQLVK NTYVL 225  
(SEQ ID NO.: 45),

15 MAQLCGL RRSRAFLALL GSLLLGVLA -1  
ADRERSIHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50  
YLTKEECLKK CATVTENATG DLATSRNAAD SSVPSAPRRQ DSEDHSSDMF 100  
NYEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 150  
20 ACMLRCFRQQ ENPPLPLGSK VVVLAGLFVM VLILFLGASM VYLIRVARRN 200  
QERALRTVWS FGD 213  
(SEQ ID NO.: 47),

ADRERSIHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50  
25 YLTKEECLKK CATVTENATG DLATSRNAAD SSVPSAPRRQ DSEDHSSDMF 100  
NYEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 150  
ACMLRCFRQQ ENPPLPLGSK VVVLAGLFVM VLILFLGASM VYLIRVARRN 200  
QERALRTVWS SGDDKEQLVK NTYVL 225  
(SEQ ID NO.: 70),

30 and

ADRERSIHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50  
YLTKEECLKK CATVTENATG DLATSRNAAD SSVPSAPRRQ DSEDHSSDMF 100  
35 NYEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 150  
ACMLRCFRQQ ENPPLPLGSK VVVLAGLFVM VLILFLGASM VYLIRVARRN 200  
QERALRTVWS FGD 213

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Ref 126  
(SEQ ID NO. : 71) .

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33: Use according to claim 19, wherein the Kunitz-type serine protease  
5 inhibitor comprises the amino acid sequence:

IHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50  
YLTKEECLKK CATV 64

(SEQ ID NO. : 4) ,

10 CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50  
YLTKEECLKK C 61  
(SEQ ID NO. : 5) ,

15 YEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 150  
ACMLRCFRQ 159  
(SEQ ID NO. : 6) ,

20 CTANAVTGPC RASFPRWYFD VERNSCNNFI YGGCRGNKNS YRSEE 150  
ACMLRC 156  
(SEQ ID NO. : 7) ,

25 IHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50  
YLTKEECLKK CATVTENATG DLATSRNAAD SSVPSAPRRQ DSEDHSSDMF 75  
NYEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 125  
ACMLRCFRQ 159  
(SEQ ID NO. : 3) ,

30 CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50  
YLTKEECLKK CATVTENATG DLATSRNAAD SSVPSAPRRQ DSEDHSSDMF 100  
NYEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 150  
ACMLRC 156  
(SEQ ID NO. : 50) ,

35 ADRERSIHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 25  
YLTKEECLKK CATVTENATG DLATSRNAAD SSVPSAPRRQ DSEDHSSDMF 75  
NYEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE 125  
ACMLRCFRQQ ENPPLPLGSK VVVLAGAVS 179

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Rule  
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(SEQ ID NO.: 1),

and

5 ADRERSIHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN  
50  
YLTKKECLKK CATVTENATG DLATSRNAAD SSVPSAPRRQ DSEDHSSDMF  
100  
NYEEYCTANA VTGPCRASFP RWYFDVERNS CNNFIYGGCR GNKNSYRSEE  
10 150  
ACMLRCFRQQ ENPPLPIIGSK 170  
(SEQ ID NO.: 52).

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34. Use according to claim 19, wherein the Kunitz-type serine protease

15 inhibitor comprises the amino acid sequence:

ADRERSIHDF CLVSKVVGRC RASMPRWYN VTDGSCQLFV YGGCDGNSNN 50  
YLTKKECLKK CATVTENATG DLATSRNAAD SSVPSAPRRQ DS 92  
(SEQ ID NO.: 8).

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35. Use according to claim 19, wherein the Kunitz-type serine protease inhibitor is glycosylated.

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25 36. Use according to claim 19, wherein the Kunitz-type serine protease inhibitor contains at least one intra-chain cysteine-cysteine disulfide bond.

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30 37. Use according to claim 19, wherein the Kunitz-type serine protease inhibitor contains at least one intra-chain cysteine-cysteine disulfide bond selected from the cysteine-cysteine paired groups consisting of CYS11-CYS61, CYS20-CYS44, CYS36-CYS57, CYS106-CYS156, CYS115-CYS139, and CYS131-CYS152, wherein the cysteine residues are numbered according to the amino acid sequence of native human placental bikunin.

25/04